

# Trichloroacetic Acid as a Biomarker of Exposure to Disinfection By-Products in Drinking Water: A Human Exposure Trial in Adelaide, Australia

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We addressed the need for a biomarker of ingestion exposure to drinking water disinfection by-products by performing a human exposure trial. We evaluated urinary excretion of trichloroacetic acid (TCAA) as an exposure biomarker using 10 volunteers who normally consume their domestic tap water. We recruited the volunteers at a water quality research laboratory in Adelaide, Australia. Participants maintained a detailed consumption and exposure diary over the 5-week study. We also analyzed tap water and first morning urine (FMU) samples for TCAA, and tap water for chloral hydrate (CH). We documented both interindividual and intraindividual variability in TCAA ingestion and urinary excretion, and both were substantial. With a TCAA-free bottled water intervention, we used creatinine-adjusted urinary TCAA levels to estimate urinary TCAA excretion half-lives for three of the participants. We observed correspondence over time between estimated TCAA excretion, calculated from TCAA + CH ingestion levels, and measured TCAA urinary excretion. This study demonstrates the merits and feasibility of using TCAA in FMU as an exposure biomarker, and reveals remaining concerns about possible alternate sources of TCAA exposure for individuals with low drinking water ingestion exposure. **Key words:** disinfection by-products, drinking water, exposure assessment, haloacetic acids, trichloroacetic acid. *Environ Health Perspect* 110:679–687 (2002). [Online 28 May 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p679-687froese/abstract.html>

The question of whether disinfection by-products (DPBs) in drinking water pose any health risk to humans has been an ongoing issue since the discovery of DPBs in 1974. Although toxicology experiments with individual DPBs are necessary to establish plausible mechanisms of toxic action, epidemiologic studies of human populations are necessary to establish whether actual DPB exposures from drinking water pose a human health risk. Such studies have historically been focused on cancer outcomes, but more recently a number of studies have addressed the possibility of adverse reproductive outcomes (1–12).

The ability of epidemiologic studies to address these health questions has been seriously limited by inadequate individual assessment of exposure to DPBs (13,14). A major prospect for improved exposure assessment for epidemiologic studies of adverse reproductive outcomes is to validate a biomarker of exposure to DPBs. Desirable characteristics of potential biomarkers of DPB exposure have been discussed by Froese et al. (15).

To date, the only DPBs evaluated for use as biomarkers of exposure have been the trihalomethanes (THMs) and the haloacetic acids (HAAs). Weisel et al. (16) found that most background breath samples from a cohort of women who had participated in the Klotz and Pyrch (8) study were nondetectable for THMs in exhaled breath. Measurable levels of THMs were obtained in post-shower breath samples, and these breath values correlated with water levels of

THMs. However, breath levels of THMs as a biomarker of exposure to DPBs do not persist for sufficient time to integrate exposure measurement over more than minutes to hours, at most. Likewise, measurable THM exposures are limited to inhalation and dermal exposure from showering or bathing. THM levels in breath will not reflect ingestion exposure because of rapid first-pass metabolism of ingested THMs in the liver. THMs in blood were demonstrated to be feasible for evaluating background THM exposure (17), but blood sampling is an invasive procedure.

The potential of two HAAs, dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA), as DPB biomarkers in urine was examined with a cross-sectional study of a cohort of 49 women, who provided 42 valid samples (16,18). That study found that DCAA was rapidly metabolized and that urine levels showed no difference between low-exposure and high-exposure groups. However, TCAA did show higher excretion levels for higher-TCAA-exposure versus lower-TCAA-exposure groups. TCAA excretion in urine did not correlate significantly with measured water concentrations of TCAA. A more detailed TCAA exposure calculation was done by accounting for the volume of water consumed, the proportion of heated water used (estimating a 39% reduction in TCAA from boiling), and the use of home water filters (estimating a 70% reduction for any filter type). With these adjustments, a

significant correlation ( $R$  of 0.73,  $n = 42$ ) was found between estimated TCAA ingestion (estimated as micrograms of TCAA consumed in the previous 48 hr) and measured TCAA excretion rate (ng/min). Given these promising results, we undertook a pilot trial to examine the viability of TCAA in urine as a biomarker of DPB ingestion exposure.

The promise of urinary TCAA as a biomarker for drinking water ingestion exposure needs to be evaluated in a longitudinal pilot study to address the temporal relationship between ingestion exposure and urinary excretion. A pilot evaluation of TCAA as a biomarker needs to characterize both interindividual and intraindividual variability of TCAA ingestion and urinary excretion, determine the persistence of TCAA excretion following ingestion (as measured by the half-life of urinary TCAA excretion), and evaluate the feasibility of TCAA in first morning urine (FMU) samples for potential use in future epidemiology studies.

We designed a pilot exposure/intervention study of approximately 5 weeks' duration to provide some insight on these issues and to guide future studies.

## Methods

**Water supply system.** Overall, the Adelaide, South Australia, metropolitan region has six major supply zones for six treatment plants, each drawing water from a particular reservoir. Because the reservoirs are affected by different watershed characteristics, the dissolved organic carbon levels of the water differ considerably as well, leading ultimately to quite different DPB concentrations and profiles among the communities in and

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around Adelaide. Table 1 outlines the characteristics of the four major water supply zones we ultimately selected.

**Recruitment of study cohort.** We solicited healthy adult volunteers from the Australian Water Quality Centre (AWQC), with the intent to recruit at least two or three participants from each of the four water supply systems we selected. We recruited a cohort of 10 volunteers, consisting of eight males and two females with ages ranging from 24 to 57 years. We expected that this cohort, associated with the AWQC, would be motivated to contribute to our research findings by cooperating with the demands of the study. The Monash University Standing Committee on Ethics in Research Involving Humans approved the study protocol, and we obtained written informed consent from participants at enrollment.

**Study design.** During the summer season in Adelaide (February 2000), we monitored participants via consumption diaries and with tap water and urine analyses for an initial period in which they consumed water from their normal tap water supply containing variable levels of TCAA, an intervention period with consumption of DPB-free bottled water, followed by a final return to normal tap water consumption (Table 2).

We classified daily fluid consumption according to cold and hot tap water and "other," with specific locations or regional locations for all fluid consumption. Hot tap water included all beverages, such as coffee and tea, that were prepared using boiled water. "Other" beverages included bottled juices, soft drinks, alcoholic beverages, and the like. Additionally, we tracked other water exposures, including showering/bathing, washing dishes, washing cars, swimming/hot tub use, and so on. We asked participants to document potential exposures to trichloroethylene (TCE) and trichloroethane from visits to dry cleaning establishments, wearing dry-cleaned clothing, or exposure to these solvents in the AWQC laboratory. Participants started the diaries 48 hr before the first urine sample collection.

Participants brought entire FMU voids and home tap water samples to the AWQC. If the participant anticipated a delay of more than 4 hr before the sample could be delivered to the lab, he or she stored the sample in a cooler with ice or in a refrigerator. We generally extracted samples for analyses within 6 hr of collection, and performed the study.

**Analytical methodology.** We performed analysis of TCAA in urine according to a modified version of U.S. Environmental Protection Agency (EPA) method 552.2 (19) and the method used by Kim and Weisel (20). We measured an aliquot of urine (40 mL) into a graduated cylinder and poured it into a 50-mL centrifuge tube. We added a surrogate standard comprised of 80  $\mu$ L of a 25  $\mu$ g/mL solution of 2,2-dichloropropionic acid and acidified the sample using 2 mL of concentrated sulfuric acid. We added approximately 12 g of sodium sulfate and 4 mL of methyl *tert*-butyl ether (MTBE) containing approximately 500  $\mu$ g/L (known concentration) of 1,2,3-trichloropropane as an internal standard. We hand-shook the samples for 8 min to ensure that the sulfate salt fully saturated the samples, and we then centrifuged them at 2,500 rpm for 15 min.

Using a Pasteur pipette, we transferred the entire solvent layer to a 10-mL glass vial, added 3 mL of acidified methanol (10%  $\text{H}_2\text{SO}_4$  in methanol), and then vortexed it for 30 sec and placed it in a 50°C heating block for 1 hr. We then added 8 mL of saturated  $\text{NaHCO}_3$  solution to the vial, after removing it from the heating block, to neutralize the acid. We added the  $\text{NaHCO}_3$  solution dropwise to avoid sputtering and solvent loss. We then transferred the solvent layer to and eluted it through a disposable activated-carbon solid-phase extraction (SPE) column (6 mL  $\times$  250 mg; Envi-Carb, Supelco, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) to reduce the organic background, as indicated by extracted color. We collected the SPE column eluate in a 2-mL autosampler vial.

We prepared tap water samples and analyzed them for HAAs in the same manner as the urine samples, with the exception that we omitted the Envi-Carb SPE step.

We analyzed the samples on a Varian 3400 gas chromatograph (GC) with a single injection (run in splitless mode) leading into two analytical capillary columns, each with an electron capture detector (ECD). The simultaneous dual column analysis [DB-1, 30 m  $\times$  0.25 mm internal diameter (i.d.), 0.25  $\mu$ m film; DB-1701, 30 m  $\times$  25 mm i.d., 0.25  $\mu$ m film] allowed for confirmation of analyte peaks. We calibrated the GC-ECD with a mixture of methyl ester HAAs. The calibration range was  $\leq$  0.1–50  $\mu$ g/L for TCAA.

These methods provided excellent performance [relative standard deviation (RSD) of 4.8% on 11 triplicate analyses of tap water and 8.5% on 17 triplicate analyses of urine] and allowed sensitive detection of TCAA in urine. Our method detection limit (MDL) for these analyses was approximately 0.2  $\mu$ g/L for tap water and 0.3  $\mu$ g/L for urine, based on three times the SD for triplicate sample analyses, averaged for triplicate sets with TCAA  $\leq$  2  $\mu$ g/L. The mean surrogate recovery for 384 analyses was 119% (median, 118%; SD, 31%); we corrected all TCAA values for recovery. We selected two urine samples at random on day 13 of the study and tested them for storage loss over 72 hr. The average absolute reduction in TCAA concentration as measured was 0.16  $\mu$ g/L, or 12%. With the average SD of analyses done at this concentration level at 0.1  $\mu$ g/L, and the fact that we analyzed the vast majority of the samples on the day we received them, we judged the loss of TCAA over 72 hr to be inconsequential for the purposes of this study.

We analyzed tap water samples for chloral hydrate (CH) using the AWQC standard test method TMS-003 (21), which is based on U.S. EPA method 551 (22). Briefly, we transferred 35 mL of tap water (collected with zero headspace and quenched with  $\text{NH}_4\text{Cl}$ ) to a glass vial, added  $\text{Na}_2\text{SO}_4$ , and extracted the samples using 2 mL of MTBE containing dibromopropane as an internal standard. We transferred sample extracts directly to 2-mL autosampler vials and analyzed them on the Varian 3400 system described above.

## Results and Discussion

**Tap water ingestion.** We easily detected TCAA in all FMU samples that we analyzed, even though most of the tap water TCAA exposure levels were lower than expected

**Table 1.** Characteristics of drinking water supply zones used in the study.

	Anstey Hill	Little Para	Happy Valley	Hope Valley
Study participants	AD1–AD4	AD5	AD6–AD8	AD9, AD10
Source water to supply reservoir	River Murray and Torrens system	Little Para system and River Murray	Onkaparinga system and River Murray	Torrens system and River Murray
Approximate average water residence time in reservoir	2 months	3 months	1 month	20 days
Free chlorine residual	1.3 mg/L	1.2 mg/L	1.5 mg/L	1.2 mg/L

All plants use the following water treatment processes: alum coagulation, flocculation, lime, anthracite-sand rapid filtration, chlorination. All plants are capable of dosing powdered activated carbon when required, and prechlorination prior to coagulation and filtration when required. Anstey Hill and Little Para are capable of caustic addition prior to coagulation. Use of River Murray is directly dependent on rainfall for the year and varies from 40% to 90% of total water supply to reservoirs.

**Table 2.** Experimental design for TCAA exposure and intervention study.

Week	Water source	FMU sample days	No. FMU samples
1	Regular tap	Tues	1
2	Regular tap	Mon–Fri	5
3	Bottled	Mon–Sun	7
4	Bottled	None	0
5	Regular tap	Mon, Wed, Fri	3

based on our study planning surveys. Relatively low TCAA ingestion exposures occurred for most participants. Detectability of TCAA in urine fulfills an important requirement for the feasibility of an exposure biomarker (15).

Figure 1 plots the temporal trends throughout the study period for participant AD2 showing the daily TCAA ingested ( $TCAA_{in}$ ) and TCAA excreted ( $TCAA_{ex}$ ). The lines connecting data points are for visualization purposes only. We examined the initial exposure phase (tap water exposure) of the study separately to examine possible relationships between  $TCAA_{ex}$  and  $TCAA_{in}$  and response variability, both inter- and intraindividual. For this aspect of the data analysis, we used data collected up to

and including 13 February 2000 (and the FMU of 14 February 2000).

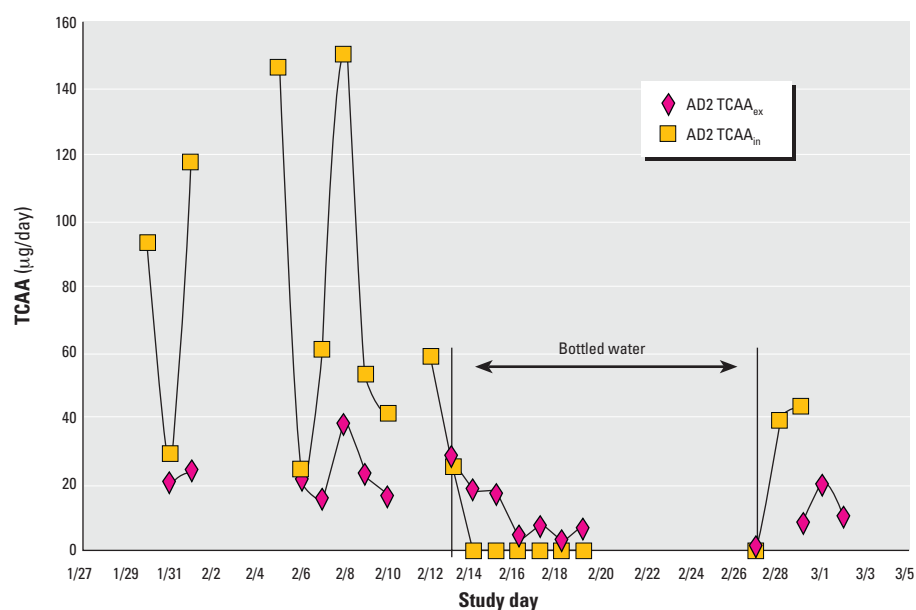
Table 3 provides a summary of results for  $TCAA_{in}$  and  $TCAA_{ex}$  showing the intraindividual variability. We calculated  $TCAA_{in}$  from the volumes of cold tap water and hot beverages consumed at the participants' homes, work, and other locations and the TCAA concentrations as measured in cold tap water at the participants' homes and at the AWQC. If participants consumed tap water or hot beverages at other locations, we specified the town or subdivision to match an approximate TCAA value for those locations where we did not take samples directly. For the hot beverages, we estimated a 35% reduction of TCAA relative to location-specific tap water to determine external exposure due to

this portion of fluid intake. We based this adjustment on our observed reduction in TCAA for water boiled for 3 min, and it is reasonably consistent with the approach used by Weisel et al. (16). However, we classified at least one-third of total fluid intake as "other," to which we can assign no TCAA exposure contribution. Because this proportion of fluid intake is so large, the potential for additional TCAA ingestion exposures to be unaccounted for is also substantial.

We calculated the  $TCAA_{ex}$  values from the measured TCAA concentrations in the urine samples, the volume of sample, and the reported time from the last previous urination to the FMU. We linearly extrapolated the hourly TCAA excretion rate for the FMU to 24 hr to estimate the daily  $TCAA_{ex}$ . Also, because the FMU samples correspond to the water consumed in the 24 hr before sampling, we adjusted the date of  $TCAA_{ex}$  back 1 day to correspond to the  $TCAA_{in}$  date. Ultimately, we defined the consumption period before sampling that affects urinary TCAA concentration by the excretion half-life relationship for that individual. Equation 1 (below) considers this definition, but for an initial comparison we adjusted  $TCAA_{ex}$  to align with  $TCAA_{in}$  1 day earlier to account for the ingestion that corresponds to the main source of the excretion for that day.

For ingestion variability, Table 3 shows that the RSD for  $TCAA_{in}$  among the 10 participants over the 12 days of tap water ingestion ranges from a low of 14% to a high of 67%. The low value corresponded to a low average  $TCAA_{in}$  (mean of 5.2  $\mu\text{g/day}$ ) compared with much higher average  $TCAA_{in}$  for the high RSD (41 and 53  $\mu\text{g/day}$ ). Average  $TCAA_{in}$  was bimodally distributed among the 10 participants, with three participants (Anstey Hill system) having mean  $TCAA_{in}$  ranging from 41 to 73  $\mu\text{g/d}$ . The other seven participants had more than 10-fold lower mean  $TCAA_{in}$ , ranging from 2.3 to 7.9  $\mu\text{g/d}$ .

We calculated  $TCAA_{in}$  from TCAA concentrations in consumed tap water and the volume consumed; therefore, the variability will depend on the variabilities of *a*) TCAA in water, which is related to the tap water location and sampling day, and *b*) volume(s) of different source waters consumed. For TCAA in water, Table 4 provides an overview of how TCAA in water varied over time and location for the tap water period of the study. TCAA concentration averages for each participant's home tap water ranged from 1.8  $\mu\text{g/L}$  to 29  $\mu\text{g/L}$ , with an average RSD of 39% over the time studied. We observed much greater variation spatially for each tap water day, with TCAA concentrations averaging from 7.7 to 20  $\mu\text{g/L}$  for any particular day, but the RSD averaged 100%. The diverse distribution network in the



**Figure 1.** Temporal trends of  $TCAA_{in}$  and  $TCAA_{ex}$  for AD2.

**Table 3.** Intraindividual variability of TCAA ingestion and excretion during 12 days of tap water ingestion (TCAA excretion estimates are based on hourly excretion rate for FMU linearly extrapolated to full 24 hr).

Participant	Water system	TCAA	Mean $\pm$ SD ( $\mu\text{g/day}$ )	RSD (%)	Minimum ( $\mu\text{g/day}$ )	Maximum ( $\mu\text{g/day}$ )
AD1	Anstey Hill	Ingestion	41 $\pm$ 27	67	3.9	94
		Excretion	5.2 $\pm$ 3.7	71	1.9	12
AD2	Anstey Hill	Ingestion	73 $\pm$ 47	64	24	150
		Excretion	24 $\pm$ 7	31	16	38
AD3	Anstey Hill	Ingestion	7.9 $\pm$ 2.6	34	5.9	14
		Excretion	1.5 $\pm$ 0.80	53	0.8	2.7
AD 4	Anstey Hill	Ingestion	53 $\pm$ 35	67	12	120
		Excretion	5.8 $\pm$ 2.3	40	2.6	9.2
AD5	Little Para	Ingestion	3.4 $\pm$ 1.2	35	1.3	5.7
		Excretion	3.1 $\pm$ 2.0	63	1.5	7.0
AD6	Happy Valley	Ingestion	5.6 $\pm$ 3.1	55	2.0	11
		Excretion	4.7 $\pm$ 0.69	15	3.9	5.7
AD7	Happy Valley	Ingestion	2.3 $\pm$ 1.3	55	1.0	5.1
		Excretion	2.4 $\pm$ 0.95	39	1.3	4.2
AD8	Happy Valley	Ingestion	3.6 $\pm$ 1.2	32	1.4	5.3
		Excretion	3.3 $\pm$ 2.1	64	1.8	7.5
AD9	Hope Valley	Ingestion	19 $\pm$ 8.6	45	4.4	32
		Excretion	4.6 $\pm$ 3.2	69	1.3	11
AD10	Hope Valley	Ingestion	5.3 $\pm$ 0.76	14	4.3	6.1
		Excretion	4.7 $\pm$ 2.25	47	2.0	7.1



greater Adelaide area, with its distinct water reservoirs for different subdivisions, has a major impact on the overall variability of the TCAA concentrations.

The other major contributors to ingestion variability were the volume of water consumed and the specific category of fluid consumed. The variability increases when the relative proportion of water from different categories changes. Participants recorded their water consumption under the categories of cold or hot water (under “hot” we assumed coffee or tea beverages), with subcategories for location of consumption: home, work, or other (suburb or location specified). A final category of “other” included all other beverages—juices, soft drinks, beer, wine, and so on. For this last category, because we did not measure TCAA concentrations, we cannot estimate TCAA exposure amount. We averaged reported consumption amounts for each beverage category over the tap water days for each individual (Table 5). We used these individual averages to determine an overall interindividual variability for the 12 days of tap water consumption before beginning the bottled water intervention. The average RSD ranged from 48% to 200%. Table 5 results show that individuals had RSDs of about 30–300% for any particular category of water consumption (volume) over the 12 tap water days of the study, clearly indicating that consistent day-to-day tap water consumption volumes cannot be assumed. This finding raises a caution about the validity of retrospective questionnaires used to reconstruct the consumption patterns over extended periods by depending on the recall of study participants.

The variability of TCAA<sub>in</sub> for AD2, for example, was caused partly by variation of TCAA in the home tap water; however, it was also largely caused by this participant consuming as little as 0.6 L to as much as 3 L of tap water at home, with the remainder of fluid consumption classified as “other.” Daily home consumption of tap water showed a variation for this individual of 48% (RSD), whereas total fluid consumption for these days varied only 25%. Other individuals showed more diverse variability because they consumed cold tap water and heated beverages at home and in the workplace, in addition to “other,” whereas AD2 recorded only home tap water and “other.”

We gained additional insight by calculating and comparing the ratios of tap water or beverages consumed relative to total liquids ingested (Table 5). Overall, we found that cold tap water consumption at home accounts for  $39 \pm 17\%$  of total consumption and at work accounts for  $7 \pm 9\%$  of total consumption. These water sources were the best characterized in this study, because we

had several tap water samples from each location. Of the remaining consumption categories, the major contributors to total consumption were hot beverages consumed at home ( $10 \pm 6\%$ ) and “other” ( $34 \pm 17\%$ ).

For excretion variability, the RSD of TCAA<sub>ex</sub> ranged from a low of 15% to a high of 71%, with a median of about 47% (Table 3). For excretion, only one participant (AD2) had a high average TCAA<sub>ex</sub> at 24  $\mu\text{g}/\text{d}$ . The other nine participants had an average TCAA<sub>ex</sub> ranging from 2.4 to 5.8  $\mu\text{g}/\text{d}$ .

Overall, these results indicate substantial interindividual variability in both daily TCAA ingestion and excretion rates. The only other published study evaluating TCAA as a biomarker of drinking water DPBs was a cross-sectional design (16,18). When urine samples and tap water samples are collected at the same time in a cross-sectional approach, the influence of daily variations and ingestion from previous days cannot be corrected. For exposure assessment from a urine sample and a water sample taken at the same time, if excretion half-lives are a matter of days, water concentrations must remain constant for several days before the sampling if we expect a meaningful evaluation of validity.

Figure 2 shows that both inter- and intraindividual variability were substantial. The error bars represent  $\pm 1$  SD for the measurements. Figure 2 shows the variability in TCAA<sub>ex</sub> compared with TCAA concentration in the tap water consumed. One participant on the Anstey Hill system (AD2) stood well apart from the rest in terms of TCAA<sub>ex</sub>. Two others, AD1 and AD4, had relatively high TCAA levels in their tap water, but their TCAA<sub>ex</sub> was substantially less than AD2, in part because of a lower level of tap water consumption. In all cases, however, the data in Figure 2 illustrate the large variability in both tap water TCAA exposure concentrations and excretion of TCAA for all participants. Faced with such variability, a

cross-sectional sampling scheme would provide only a limited basis to evaluate and account for interindividual variability, and no basis to evaluate or account for intraindividual variability of urinary TCAA.

We consistently found the highest tap water ingestion exposures in the Anstey Hill system (participants AD1–AD4). In this distribution system, we observed substantial daily variation of the TCAA concentrations in tap water at each of the homes of the four participants (Table 4), making questionable any assumption of consistent water quality from day to day. The variation from location to location, most noticeable between AD3 and the three other participants in the Anstey Hill system, also shows the difficulty of generalizing exposures on the basis of assumptions about consistent water quality at different distribution system locations served by the same treatment plant source.

To evaluate further the interindividual variability for those who were most substantially exposed to TCAA ingestion via tap water exposure, we analyzed the differences among the Anstey Hill participants AD1–AD4 (Table 6). These data show that daily RSD among those four participants served by the same treatment plant ranged from 54% to 100% for TCAA<sub>in</sub> and from 54% to 150% for TCAA<sub>ex</sub>.

CH, which has been used extensively as an anesthetic in clinical practice, is metabolized to TCAA (23). Consequently, we measured CH in the tap water throughout the study, and it appears to have a variable relationship with TCAA<sub>in</sub> or TCAA<sub>ex</sub>; that is, CH did not correlate well with TCAA in water, nor did it correlate significantly with urinary TCAA. However, we considered the contribution of CH to TCAA<sub>ex</sub> in accounting for the total potential for urinary TCAA<sub>ex</sub>, to evaluate the correspondence of excretion with ingestion as well as

**Table 4.** Variability in tap water TCAA ( $\mu\text{g}/\text{L}$ ) for different homes, distribution systems, and sample dates.

Date	Anstey Hill				Little Para	Happy Valley			Hope Valley		Mean $\pm$ SD	RSD (%)
	AD1	AD2	AD3	AD4	AD5	AD6	AD7	AD8	AD9	AD10		
1 Feb	47	49	5.1	52	2.6	5.1	3.4	4.3	10	NA	20 $\pm$ 22	110
7 Feb	35	41	7.5	39	2.2	3.1	2.5	4.1	25	3.3	16 $\pm$ 17	100
8 Feb	43	42	16	39	2.0	3.1	2.1	3.8	NA	3.1	17 $\pm$ 19	110
9 Feb	25	22	9.4	26	1.2	NA	1.6	2.5	17	2.2	12 $\pm$ 11	90
10 Feb	26	19	11	22	1.1	2.0	1.7	2.9	NA	1.7	9.7 $\pm$ 10	110
11 Feb	16	17	5.8	17	1.2	1.4	1.3	2.3	NA	NA	7.7 $\pm$ 7.5	97
14 Feb	17	18	6.4	19	1.5	2.0	1.9	2.2	15	2.3	8.5 $\pm$ 7.6	90
28 Feb	16	22	2.4	13	1.7	2.4	2.6	2.1	11	3.3	7.7 $\pm$ 7.3	95
1 Mar	28	29	1.5	26	2.5	2.8	3.0	3.8	5.7	4.7	11 $\pm$ 12	110
3 Mar	32	34	NA	29	1.9	3.4	3.0	3.5	NA	NA	15 $\pm$ 13	100
Mean	28	29	7.2	28	1.8	2.8	2.3	3.1	14	2.9		100
SD	11	11	4.5	12	0.5	1.1	0.7	0.8	6.6	1.0		
RSD (%)	39	39	62	42	30	38	30	27	48	33	39	

NA, not analyzed. Temporal variability for each site is indicated in the lower rows by average value for the days sampled and the relative percentage deviation. Spatial variability across sites for each day sampled is represented in the right-hand columns by the average value and the relative percentage deviation.

the proportion of potential TCAA<sub>ex</sub> that we actually observed.

**Bottled water intervention.** In the third week of the exposure study, we asked participants to switch to DPB-free bottled water for all hot and cold water ingestion. We made bottled water and a dedicated kettle available in the workplace cafeteria so that boiled

beverages could be prepared using the DPB-free water. In the time-trend plots in Figure 1, the decrease in TCAA<sub>ex</sub> can be seen to lag the introduction of bottled water by a few days.

**Elimination half-life estimation.** There has been limited research directly on the pharmacokinetics of TCAA excretion, with most of the literature focused on TCAA as

a metabolite of either CH or TCE. Elimination half-life estimates for plasma TCAA ranged from 70 hr (2.9 days) to 120 hr (5 days) based on oral ingestion of 6.25–40 mg/kg CH (24,25). These CH doses correspond to levels 2,000 to more than 10,000 times higher than realistic TCAA drinking water exposure levels.

**Table 5.** Average volumes and relative ratios of fluids from each category and location from participant consumption journals.

Category location	Volumes of fluids consumed in each category and location (mL)								Ratios of beverages consumed to total volume						
	Cold			Hot			Other	Total	Cold (totals %)			Hot (totals %)			Other total (%)
	Home	Work	Other	Home	Work	Other			Home	Work	Other	Home	Work	Other	
AD1															
Mean	975	0	400	0	0	75	1,700	3,150	31	0	13	0	0	2	54
SD	575	0	550	0	0	125	725	761	18	0	17	0	0	4	23
RSD (%)	59		140			170	43	24							
n	10	10	10	10	10	10	10	10							
AD2															
Mean	2,150	0	0	0	0	0	1,400	3,550	61	0	0	0	0	0	39
SD	1,050	0	0	0	0	0	300	900	30	0	0	0	0	0	8
RSD (%)	49						21	25							
n	10	10	10	10	10	10	10	10							
AD3															
Mean	950	350	0	100	325	0	800	2,500	38	14	0	4	13	0	32
SD	375	325	0	125	325	0	175	300	15	13		5	13	0	7
RSD (%)	39	93		130	100		22	12							
n	10	10	10	10	10	10	10	10							
AD4															
Mean	1,000	600	0	475	275	0	575	2,900	34	21	0	16	9	0	20
SD	525	550	0	300	250	0	150	275	18	19	0	10	9	0	5
RSD (%)	53	92		63	91		26	9							
n	10	10	10	10	10	10	10	10							
AD5															
Mean	1,150	200	0	425	225	0	500	2,500	46	8	0	17	9	0	20
SD	500	250	0	225	225	0	475	575	20	10	0	9	9	0	19
RSD (%)	43	130		53	100		95	23							
n	10	10	10	10	10	10	10	10							
AD6															
Mean	1,400	300	50	0	25	0	575	2,350	60	13	2	0	1	0	24
SD	400	425	100	0	75	0	375	575	17	18	4	0	3	0	16
RSD (%)	29	140	200		300		65	24							
n	10	10	10	10	10	10	10	10							
AD7															
Mean	400	10	25	350	350	150	393	1,650	24	1	2	21	21	9	24
SD	200	25	50	200	375	350	287	300	12	2	3	12	23	21	17
RSD (%)	50	250	200	57	107	233	73	18							
n	10	10	10	10	10	10	10	10							
AD8															
Mean	350	100	25	225	200	0	875	2,025	17	5	1	11	10	0	43
SD	275	125	50	150	200	0	575	600	14	6	2	7	10	0	28
RSD (%)	79	130	200	67	100		66	30							
n	10	10	10	10	10	10	10	10							
AD9															
Mean	525	275	175	425	150	125	1,350	2,925	18	9	6	15	5	4	46
SD	400	350	375	475	200	150	350	625	14	12	13	16	7	5	12
RSD (%)	76	130	210	110	130	120	26	21							
n	10	10	10	10	10	10	10	10							
AD10															
Mean	1,550	175	0	450	200	0	1,225	3,500	44	5	0	13	6	0	35
SD	275	475	0	150	225	0	1075	925	8	14	0	4	6	0	31
RSD (%)	18	270		33	110		88	26							
n	7	7	7	5	7	7	7	7							
Average	1,050	200	75	250	175	25	950	2,700	37	8	2	10	7	2	34
Mean SD	450	250	125	175	200	75	450	575	17	9	4	6	8	3	17
Interindividual RSD (%)	43	125	170	70	110	300	47	21	44	120	170	66	110	190	49

n = Number of days of tap water exposure for which ingestion data is available. Each participant recorded ingested fluid volumes on daily journal sheets and provided those sheets with each FMU and tap water sample. Volumes were recorded to the nearest 25 mL. All calculations given in this table were derived from these data and have been rounded to the nearest 25 mL. Volume averages, SD, and RSD are listed for each individual in the left-hand section of the table and indicate intraindividual variation over the 10 days of tap water consumption. At the bottom of this section, overall averages and SDs indicate interindividual variability across all 10 participants for this study period. These numbers are derived from the individual averages and the individual SD values. For each participant, ratios of average volumes in each category and location versus the average total consumption volume are provided in the right-hand section. Below each average percentage contribution to the total is the ratio of the SD to the average total volume; therefore, the relative proportion of the various categories and locations of fluids consumed can be written as, for example, 31 ± 18%.

Bruning et al. (25) quote an elimination half-life of 100 hr (4.2 days) for TCAA as a metabolite of TCE, but they do not provide any experimental details or any reference citation. Muller et al. (26,27) reported an elimination half-life of 50.5 hr (2.1 days) for an oral dose of 3 mg/kg of TCAA. Much earlier, Paykoc and Powell (28) reported TCAA experiments for which Schultz (29) has calculated elimination half-lives of 99 hr (4.1 days), 76 hr (3.2 days), and 74 hr (3.1 days) for three volunteers given intravenous injection doses of 37.3, 60.2, and 28.1 mg/kg of TCAA, respectively. These doses are  $\geq 10,000$  times higher than realistic TCAA drinking water exposure levels.

Because most of the tap water exposures to TCAA were lower than expected in this pilot study, only three participants (AD1, AD2, AD6) provided results that were useful for estimation of TCAA urinary elimination half-life ( $t_{1/2}$ ). In each case, we divided the TCAA concentration measured in FMU samples by the creatinine concentration for these samples to correct for variations in FMU volume. We plotted this creatinine-normalized TCAA concentration ( $\mu\text{g TCAA/g creatinine}$ ) against time in days, with time zero (13 February) being the start of the bottled water intervention. We evaluated the logarithm of the creatinine-normalized TCAA urinary excretion for a linear fit against time (corresponding to an exponential decay). Figure 3 shows the urinary TCAA die-off curve for AD1, suggesting an elimination half-life of 3.67 day. This data set is not extremely convincing, with an  $R^2$  of only 0.45. The poor fit is mainly caused by the high value for day 1, which was suspiciously high in TCAA and low in creatinine relative to urinary volume. We have chosen to leave this point in the analysis with the foregoing qualifier, acknowledging the poor quality of fit.

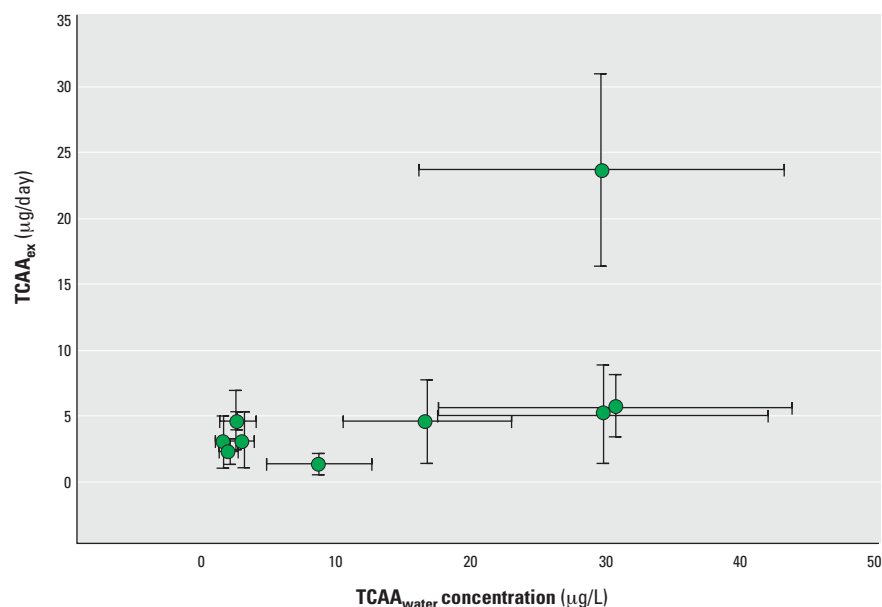
Figure 4 shows the urinary TCAA die-off curve for AD2, the highest exposed participant, suggesting an elimination half-life of 2.3 days. This data set is more convincing, with an  $R^2$  of 0.74 for the exponential decay curve. Finally, Figure 5 shows the urinary TCAA die-off curve for AD6, suggesting an elimination half-life of 2.9 days and an  $R^2$  of 0.70 for the exponential decay curve. Although the number of data points for each of these curves is limited, these data are the first reported for TCAA elimination half-life measured for TCAA concentrations low enough to be relevant to drinking water exposure and for TCAA administered by drinking water ingestion. The values observed are fully consistent with the few human values reported in the literature for high exposure and metabolite-generated TCAA elimination volunteer studies. This consistency suggests that urinary TCAA elimination is not likely

to be saturated by any plausibly high level of TCAA exposure via drinking water.

**Correspondence of excretion with ingestion.** The longitudinal design of our study also allowed us to explore, albeit with limited numbers of data points, whether there is any correspondence between ingestion and excretion of TCAA. Ultimately, confidence in a relationship between ingestion and excretion must be established for urinary TCAA to provide any value for DPB exposure assessment.

We limited the evaluation of individuals over time to those who provided reasonable data on elimination half-life. We made this choice because we needed to account for the

influence of prior exposure carrying over to observed excretion. The definition of urinary elimination half-life, (e.g., 2–3 days) means that after this time period only half of a batch of TCAA ingested will have been excreted. After two half-lives have passed, up to 25% of the total available excretion from the prior exposure would still contribute toward excretion on the subsequent day. We used a running average of ingestion that accounts for TCAA + CH ingested on previous days, adjusted according to the exponential die-off, to generate a calculated upper bound to TCAA excretion (assuming 100% of ingested CH is metabolized to TCAA and all internal



**Figure 2.** TCAA<sub>ex</sub> versus TCAA concentration in water for all participants for tap water exposure (12 days). Error bars represent  $\pm 1$  SD.

**Table 6.** Interindividual variability during 12 days of tap water TCAA ingestion—Anstey Hill water distribution system (participants AD1–AD4).

Date	Type	Mean TCAA $\pm$ SD ( $\mu\text{g/day}$ )	RSD (%)	Minimum ( $\mu\text{g/day}$ )	Maximum ( $\mu\text{g/day}$ )
30 January	Ingestion	61 $\pm$ 52	86	7.2	120
31 January	Ingestion	36 $\pm$ 27	74	5.9	70
	Excretion	8.1 $\pm$ 8.7	110	1.0	21
1 February	Ingestion	54 $\pm$ 49	91	8.3	120
	Excretion	15 $\pm$ 8.1	54	8.6	24
5 February	Ingestion	82 $\pm$ 61	75	7.2	150
6 February	Ingestion	43 $\pm$ 30	70	13	78
	Excretion	10 $\pm$ 7.7	74	2.2	21
7 February	Ingestion	48 $\pm$ 38	80	6.5	94
	Excretion	8.6 $\pm$ 6.5	75	3.2	16
8 February	Ingestion	61 $\pm$ 61	100	14	150
	Excretion	12 $\pm$ 18	150	0.8	38
9 February	Ingestion	26 $\pm$ 21	81	6.3	53
	Excretion	8.4 $\pm$ 10	120	2.7	24
10 February	Ingestion	20 $\pm$ 16	79	6.2	42
	Excretion	5.7 $\pm$ 7.4	130	1.6	17
12 February	Ingestion	26 $\pm$ 26	100	3.9	59
13 February	Ingestion	24 $\pm$ 13	54	6.4	37
	Excretion	9.2 $\pm$ 13	140	0.8	29
Range	Ingestion	20–82	54–100	3.9–13	37–150
	Excretion	8.1–15	54–150	0.8–8.6	16–38

TCAA is 100% excreted in urine). We made the calculation according to:

$$\text{TCAA}_{\text{ex}(c)} = \frac{1}{m} \sum_{t=0}^{m-1} (\text{TCAA} + \text{CH})_{\text{in}} e^{-k(t+0.333)}, \quad [1]$$

where  $\text{TCAA}_{\text{ex}(c)}$  is the estimated maximum potential urinary excretion of TCAA (micrograms per day);  $(\text{TCAA} + \text{CH})_{\text{in}}$  is the calculated ingestion of TCAA + CH (micrograms per day) for day  $m$ ;  $k = 0.693/t_{1/2}$ ;  $t_{1/2}$  is the excretion half-life estimated for each individual or the mean of all those measured where we did not determine an individual  $t_{1/2}$ ;  $t$  in days is incremented from 0 to  $m-1$ ;  $m$  is the integer value nearest to twice the individual  $t_{1/2}$ ; and  $t = 0$  corresponds to the day of a measured  $\text{TCAA}_{\text{ex}(m)}$  sample.

The molecular weights of TCAA and CH differ by only 1%, so we simply added

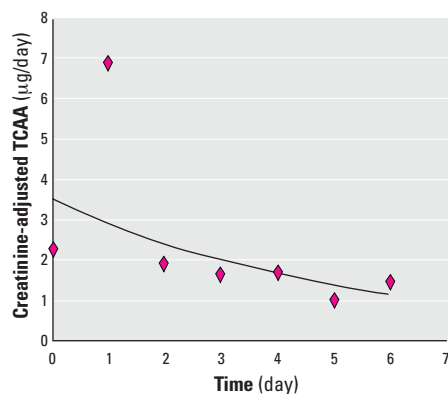
their mass concentrations for calculations of potential generation of TCAA in urine.

We assessed time series correspondence between measured TCAA excretion [ $\text{TCAA}_{\text{ex}(m)}$ ] and the calculated upper bound for excretion [ $\text{TCAA}_{\text{ex}(c)}$ ] for AD2 and AD6 (Figures 6 and 7, respectively). Figure 6 for AD2 shows a reasonable correspondence over the duration of the study.  $\text{TCAA}_{\text{ex}(m)}$  was below  $\text{TCAA}_{\text{ex}(c)}$  during the period of tap water ingestion. During the bottled water intervention,  $\text{TCAA}_{\text{ex}(c)}$  was slightly above or below  $\text{TCAA}_{\text{ex}(m)}$  in the beginning, with measured excretion levels rising above those predicted from the residual carryover from the ingestion of TCAA + CH that occurred before the bottled water intervention. We also found this trend in Figure 7 for AD6, where trends also generally match between  $\text{TCAA}_{\text{ex}(m)}$  and  $\text{TCAA}_{\text{ex}(c)}$ . Observations for both participants are consistent with a low-level source of  $\text{TCAA}_{\text{ex}(m)}$  that was independent of the low

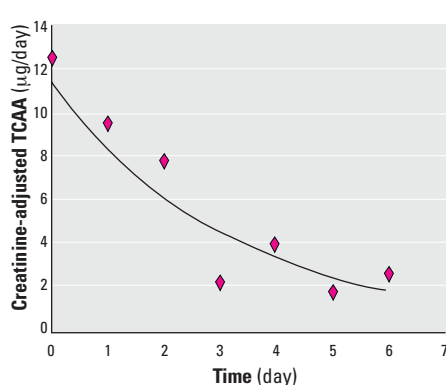
levels of  $\text{TCAA}_{\text{in}}$  that occurred with the onset of the bottled water intervention.

Table 7 summarizes the ratios of  $\text{TCAA}_{\text{ex}}$  to  $\text{TCAA}_{\text{in}}$ , based on comparison of  $\text{TCAA}_{\text{ex}}$  with the  $(\text{TCAA} + \text{CH})_{\text{in}}$ , averaged over all exposure days, for each participant. These ratios ranged from 0.12 to 0.67. We calculated these data using Equation 1, applying the mean observed half-life of 3 days for the three participants (AD1, AD2, and AD6) to all other participants and using Equation 1 to calculate a contribution to ingestion for the previous 3 days.

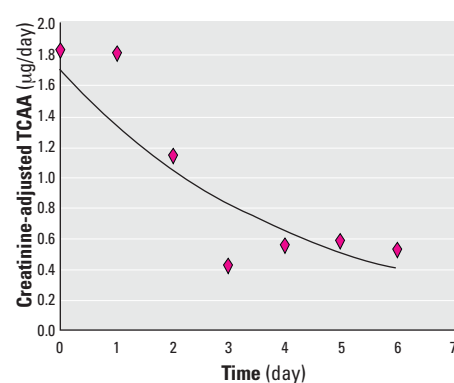
The observation that none of the participants'  $\text{TCAA}_{\text{ex}}$  levels dropped below the MDL after completion of the DPB-free bottled water intervention needs to be evaluated in relation to expected levels of  $\text{TCAA}_{\text{ex}}$  after this duration of 2 weeks of TCAA-free water ingestion. After 5 days on bottled water, AD1 and AD6 would have been expected to demonstrate  $\text{TCAA}_{\text{ex}}$  levels of 40% and



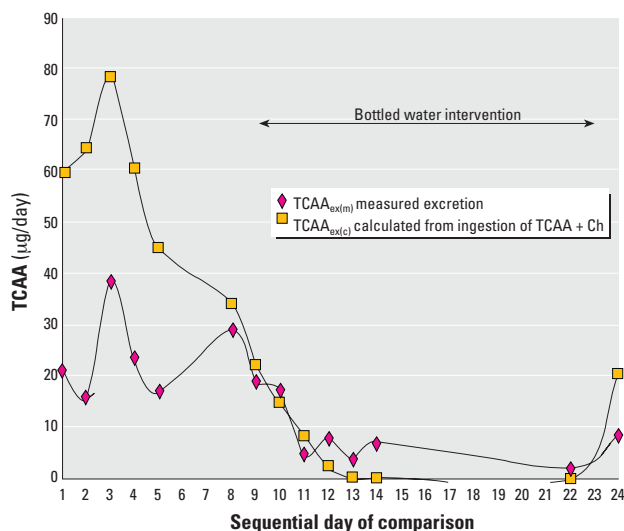
**Figure 3.** TCAA elimination half-life estimation based on bottled water intervention for AD1 using creatinine-adjusted TCAA concentration in FMU.



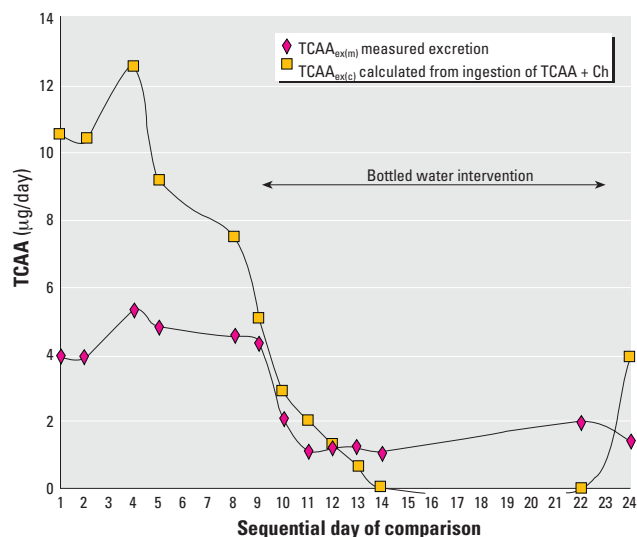
**Figure 4.** TCAA elimination half-life estimation based on bottled water intervention for AD2 using creatinine-adjusted TCAA concentration in FMU.



**Figure 5.** TCAA Elimination half-life estimation based on bottled water intervention for AD6 using creatinine-adjusted TCAA concentration in FMU.



**Figure 6.** Correspondence over the duration of the study of measured TCAA excretion with half-life-adjusted excretion calculated from TCAA + CH ingestion in tap water for AD2.



**Figure 7.** Correspondence over the duration of the study of measured TCAA excretion with half-life-adjusted excretion calculated from TCAA + CH ingestion in tap water for AD6.



**Table 7.** Proportion of TCAA excreted in urine ( $TCAA_{ex}/(TCAA + CH_{in})$ ) from a half-life-adjusted estimate of excretion potential based on tap water TCAA + CH ingestion.

Participant	Mean	SD	RSD (%)	Minimum	Maximum
AD1	0.14	0.12	88	0.05	0.33
AD2	0.33	0.26	76	0.23	0.83
AD3	0.17	0.09	56	0.07	0.29
AD4	0.12	0.03	24	0.09	0.17
AD5	0.60	0.51	85	0.24	1.50
AD6	0.39	0.16	42	0.24	0.66
AD7	0.67	0.27	40	0.43	1.04
AD8	0.36	0.16	44	0.20	0.62
AD9	0.28	0.29	100	0.07	0.80
AD10	0.34	0.13	38	0.20	0.45

The excretion rate ( $\mu\text{g/day}$ ) was estimated based on FMU TCAA concentrations and urinary excretion rate ( $\mu\text{g/day}$ ) adjusted to 24 hr. The TCAA + CH ingestion, estimated to be available for excretion, was calculated according to Equation 1, using  $t_{1/2} = 3$  days, which was a mean of the only three half-lives measured among the 10 participants. The calculation for these estimates presumed 100% excretion of TCAA + CH ingested and was done for up to eight individual days per participant between 30 January and 15 February when TCAA was measured, working back for the previous 3 days (~1 half-life).

30% of the tap water “steady-state” excretion value, respectively, according to their half-life estimates. Observed excretion levels were close to these predicted values, at 40% and 25%, respectively.

After 14 days on bottled water, AD2 would have been expected to demonstrate TCAA<sub>ex</sub> levels of 2% of the tap water “steady state” excretion value according to the applicable half-life estimate. Observed excretion levels were substantially higher, 7.5%, than would be predicted from the residual TCAA excretion washout curve. For the remaining participants for whom half-lives could not be determined, we assumed a half-life of 3 days, leading to an expectation of finding TCAA<sub>ex</sub> levels of 4% of the tap water “steady state” excretion values after 14 days on TCAA-free bottled water. The values observed ranged from 18% to an outlier value of 270%, with a median of 50%. These observations also suggest that other sources of TCAA exposure may have occurred for some participants, indicating the need for further investigation of low-level sources of TCAA from other beverage or food sources.

The viability of TCAA as a biomarker in a large-scale epidemiology study is an important consideration. Collection of FMU samples from participants was not a problem among our volunteers. Weisel et al. (16) obtained FMU samples from 47 of 49 participants of their cross-sectional study, of which 42 were accepted as valid samples, for an 86% compliance rate. Likewise, the logistics of FMU and tap water sample collection are likely manageable. The main issue will be analytical laboratory capacity and analytical costs, both of which will be a function of the analytical method used. Because these features will likely be limiting constraints on the feasibility of using FMU samples for TCAA analysis as a biomarker of DPB exposure, we need to reduce the analytical time and cost of TCAA analyses (14,30).

## Conclusions

In this human exposure and intervention study, we found considerable inter- and intraindividual variability in both TCAA ingestion and excretion. Such variability seems likely to occur for other individual DPBs, raising questions about generalizations of DPB exposures from routine water quality monitoring data, as has been commonly done for epidemiologic studies. A major contribution to the observed variability is the variability in source and volume of water consumed for each individual and across all individuals in the study.

Three study participants demonstrated an apparent excretion half-life of TCAA ranging from 2.3 to 3.7 days. This urinary excretion half-life is considerably longer than any of the THM biomarkers and is consistent with expectations for TCAA from the limited literature-based human pharmacokinetic data obtained using much higher (by 3 to 4 orders of magnitude) nondrinking water TCAA exposures.

TCAA can be readily detected in urine with the analytical methods adapted for this study. This capability revealed that urinary TCAA excretion levels were generally low after prolonged exposure to TCAA-free bottled water. However, the failure of TCAA to decline to expected low levels, most notably in those participants with the lowest TCAA ingestion exposure before the bottled water intervention, raises concerns about the specificity of urinary TCAA levels as a marker of drinking water ingestion.

TCAA in urine was sufficiently stable to allow monitoring in this study with rapid turnaround of analyses. Urinary sampling for TCAA is relatively noninvasive, particularly when FMU samples are used. Although this study has established that monitoring urinary TCAA is technically feasible, analytical costs and analytical resources would likely be a serious constraint for a larger scale study. Despite several qualifiers, TCAA remains the most

promising prospect for a biomarker of ingestion exposure to DPBs in drinking water.

The study results clearly indicate the need for more closely controlled exposure studies to provide greater understanding of TCAA intake sources, TCAA urinary excretion, and inter- and intraindividual variability by avoiding the influence of large differences in fluid consumption patterns that occurred in this trial.

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